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[1]H.S. Swartzwelder, Center for Alcohol Studies, Univ. of N.C. Medical Center, Chapel Hill, NC 27514, [2] Dept. of Psychology, St. Louis Univ., St. Louis MO; [3]NTD, (MD-74B), HERL, U.S. Environmental Prot. Agency, RTP,	11. CONTRACT/GRANT NO.

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Depressed Excitability and Integrated EEGs Following Hippocampal Afterdischarges

H. SCOTT SWARTZWELDER,[1] STEVEN T. WEGENER,[2]
CRAIG T. JOHNSON AND ROBERT S. DYER[3]

Department of Psychology, Towson State University, Baltimore, MD 21204

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Postictal depression Hippocampus Afterdischarge Afterdischarge threshold Neuroexcitability

IN VIEW OF their increasing use as tools in various areas of brain research [3, 4, 6, 9, 10, 11], it is appropriate to examine carefully some of the intrinsic properties of hippocampal (HPC) afterdischarges (ADs). In most previous experiments, investigators have chosen a single high stimulus intensity and applied it to all subjects in order to ensure that all subjects had ADs [2, 6, 13]. Although this procedure is convenient, it has the ultimate effect of placing each animal's eliciting stimulus intensity in a unique relationship with its AD threshold (since variation does exist between animals with regard to AD thresholds). In a recent report [5], the AD duration and postictal depression (PID) duration were measured following stimulus intensities 115% and 400% of AD threshold. The low (115%) stimulus intensity resulted in long ADs and short PIDs relative to the ADs and PIDs produced by high (400%) stimulus intensity. This finding indicates that it is probably not appropriate to use a single stimulus intensity to elicit ADs if between animal durations of either the AD or PID are of interest. In that report, the relationship between stimulus intensity and postictal neuroexcitability, which may or may not be related to the PID, was not addressed. It is important to investigate this relationship, because some of the interesting phenomena reported (e.g. kindling and self-sustaining seizures, [13]) may depend upon alterations in neuroexcitability which are secondary to the high stimulus intensities employed.

The PID is observed as a flattening of the EEG following an AD. The reduced amplitude of EEG waves during the PID presumably reflects reduced neural activity in the region of the recording electrodes, but it is not clear to what extent the HPC is refractory to subsequent AD-inducing stimuli during the PID, or if PID duration is correlated with AD susceptibility at all. Several previous studies provide some clues. Taber *et al.* [13], in an important study demonstrating self-sustaining seizures, reported that during the HPC PID which follows ADs in mice, electrical stimulation 8×threshold failed to elicit a subsequent AD. Herberg and Watkins [7] reported that an AD can elevate the threshold for a subsequent AD for a period of several hours, while Mucha and Pinel [8] found that high intensity stimulation elevated the AD threshold for as long as a week. None of these studies attempted to directly address the time course of AD susceptibility following an initial AD, although several have addressed the time course of alterations in the characteristics of seizures or ADs following antecedent stimuli. One way of making this assessment is to evaluate the time required to elicit a second AD with a threshold stimulus. Previous work has shown that stimuli well above AD threshold produce long PIDs [5]. The present study used several different stimulus intensities (relative to threshold) to explore the relationship between PID duration and AD susceptibility.

[1]Send reprint requests to H. S. Swartzwelder, Center for Alcohol Studies, University of North Carolina Medical Center, Chapel Hill, NC 27514.
[2]Department of Psychology, St. Louis University, St. Louis, MO.
[3]Neurotoxicology Division (MD-74B), Health Effects Research Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, NC 27711.

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METHOD

Fifteen male hooded Long-Evans rats purchased from Charles River and weighing 300–350 g at the time of implantation served as subjects. Bipolar electrodes with tips separated by 0.50 mm in the vertical plane were constructed by twisting two insulated 0.25 mm nichrome wires.

The electrodes were aimed at the left dorsal HPC (Bregma −2.7 mm, lateral 2.7 mm, ventral from skull surface 3.8 mm, incisors 5 mm above earbars) according to standard stereotaxic procedures under sodium pentobarbital (50 mg/kg) anesthesia. Each animal was allowed at least 14 days for recovery before testing.

The Plexiglas testing chamber (31×31×31 cm) was housed in a Faraday cage. Electrographic activity was conducted from the bipolar electrode via shielded Microdot "mininoise" cables to a Grass model 7B polygraph for amplification and display. A Grass model S-88 stimulator and 2 PSIU-6 constant current converters were used to produce 2 sec trains of 0.3 msec biphasic constant current square waves, delivered at 50 Hz through the recording electrode. A switching circuit isolated the polygraph amplifiers during the delivery of stimulation.

At the beginning of each recording session, each animal had at least 60 sec of baseline electrographic activity monitored prior to stimulation. AD thresholds for all animals were established according to the method described by Pinel *et al.* [9]. This method involved administering at 60-sec intervals an ascending series of intensities (6, 8, 10, 14, 22, 30, 50, 70, 90, 110, and 130 μA) until an AD of at least 6 sec duration was induced.

Four days later, animals were divided into three equal groups matched according to AD thresholds. Groups 1, 2, and 3 received stimulation at 115, 200, and 800% of threshold, respectively. ADs (AD1) were induced in all cases and, within 2 sec of the termination of the AD, subsequent stimulation was begun at 115% of threshold level for all animals and was continued at 30-sec intervals until a second AD (AD2) was induced. Durations of the ADs were measured in sec, and the number of stimulations necessary for production of AD2 was recorded. The data were analysed by one-way analyses of variance.

EEGs were analysed according to the rules of Dyer *et al.* [4,5], and F-tests for repeated measure designs were applied to the resulting data for AD durations, and number of stimuli necessary to elicit AD2. At the end of the experiments, all animals underwent cardiac perfusion with saline and Formalin. Electrode placements were varified by an analysis of frozen coronal sections stained with cresylviolet.

RESULTS

Analysis of the brain sections revealed that all electrode tips were either in the CA1-CA2 or area dentata regions of the dorsal hippocampal formation. The duration of AD1 decreased as the stimulus intensity used to induce it increased (see Fig. 1), thus confirming and extending the previously described [5] relationship for intensities 115% and 400% of threshold. The duration of AD2 as a function of the stimulus intensity used to induce AD1 is also represented in Fig. 1. As the stimulus intensity eliciting AD1 increased, so did the duration of AD2, $F(2,12)=8.69$, $p<0.01$, in direct opposition to the relationship between stimulus intensity and AD1 duration. Figure 2 illustrates that as initial stimulus intensity increased, the number of subsequent near-threshold stimuli necessary to induce a second AD (AD2) decreased, $F(2,18)=5.49$, $p<0.025$.

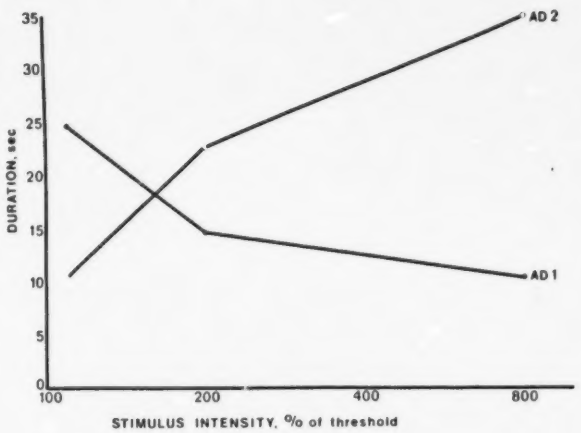

FIG. 1. Effect of AD1 stimulus intensity upon the duration of AD1 and AD2. All AD2s were elicited by stimuli 115% of threshold while AD1s were elicited by intensities indicated on the abscissa.

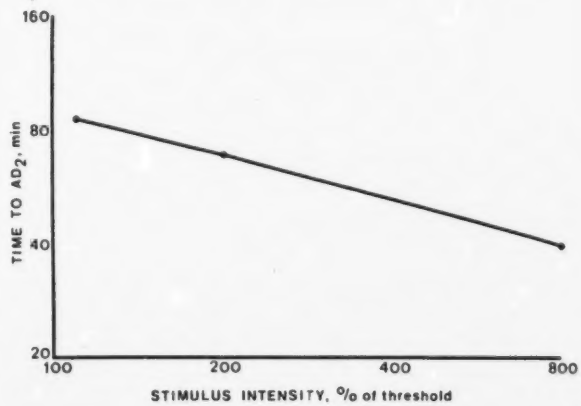

FIG. 2. Effect of stimulus intensity upon the duration of depressed hippocampal neuroexcitability.

DISCUSSION

These results provide evidence that the susceptibility of the HPC to a second AD (AD2) is sensitive to changes in the stimulus intensity used to induce the preceding AD (AD1). As stimulus intensity used to elicit AD1 increased, the duration of AD1 decreased while AD2 durations (which were always elicited by a 115% of threshold stimulus) increased.

Since stimuli which give rise to long PIDs [5] are the same as those after which fewer postictal near-threshold stimuli are necessary for AD2 production (i.e. both are high intensity), it can be concluded that the EEG flattening that defines the PID is not directly related to AD susceptibility. The fact that no AD2s were actually triggered during the PID following AD1 is in agreement with the findings of Taber *et al.* [13]

in mice. The fact that lethal self-sustaining seizures occur in mice whose AD2s occurred early (i.e., fewer stimuli to AD2) [13] may indicate that the stimulus intensity used to elicit the ADs was more elevated with respect to threshold than it was for animals developing non-lethal self-sustaining seizures.

Since low stimulus intensities give rise to long ADs and high stimulus intensities give rise to short ADs [5], it is conceivable that postictal phenomena may vary primarily as a result of AD duration and only secondarily as a function of stimulus intensity. Some evidence exists to support the notion that this is not true. Type II ADs are usually shorter than type I ADs, and yet type II ADs have no PID [5]. Long ADs are associated with short PIDs and long periods of hypoexcitability, while short ADs are associated with long PIDs and short periods of hypoexcitability. One possible explanation of these seemingly paradoxical phenomena is that postictal neural activity and postictal neuroexcitability are mediated by separate mechanisms.

A remarkable parallel exists between the time course of depressed susceptibility to ADs after AD1 and the period of neuronal subsensitivity of CA1 pyramidal cells to acetylcholine (ACh) which occurs after an AD1 [1]. According to Burchfiel *et al.* [1], an AD elicited by a stimulus (of unspecified relationship to AD threshold) is followed by a period of ACh subsensitivity in individual CA1 neurons lasting usually between 30 and 60 min. Following this period of subsensitivity is a period of supersensitivity which lasts at least another 60 min and probably longer. ADs elicited during the period of supersensitivity were longer than AD1s, a finding consistent with the present findings that AD2s are longer than AD1s when they occur at the end of the AD subsensitive period. The present findings indicate (1) the relevance of Burchfiel's single cell subsensitivity in the anesthetized rat to more macroelectrical phenomena in the unanesthetized animal; (2) a quantitative measure of the duration of the subsensitive period; and (3) a demonstration that the duration of the subsensitive period is shorter when the initial AD eliciting stimulus is more intense.

There have been several previous reports of post-seizure inhibition of subsequent motor seizures in kindled rats [8,12], and it is important to discriminate between them and the present report. Sainsbury *et al.* [12] kindled rats by hippocampal stimulation until 10 consecutive motor seizures had been elicited. The subsequent post-seizure inhibition testing consisted of spacing stimuli from 10–70 min after an initial seizure-eliciting stimulus. AD2s were always elicited, even at the 10 min interval, and mild motor seizures were elicited in most rats by 30 min. Since stimulus intensity was constant (400 μA), and thresholds were not determined, it is impossible to compare directly their results with those of the present study. Their findings that ADs could be elicited 10 min after a kindled motor seizure might reflect both the high intensity of the initial stimulus and the high intensity of the second stimulus. Preliminary results (unpublished) from our laboratory indicate that stimuli 400% of threshold elicit ADs 15 min after a threshold AD1 in about 90% of cases.

Mucha and Pinel [8], using kindled amygdaloid seizures, reported results quite different from the present findings. When a stimulus at approximately AD threshold was followed 100 sec later by a 5×threshold stimulus, AD2 was about twice as long as AD1. When AD1 was produced by a stimulus about 200% of threshold, AD1 duration increased and AD2 duration decreased. These differences may indicate a different relationship between stimulus intensity and AD properties in the hippocampus and amygdala.

In conclusion, the present paper offers further evidence for the importance of stimulus intensity in studies of ADs, kindling and self-sustaining seizures. The present paper also points out a relationship between AD susceptibility and neurochemical parameters which will probably be important in understanding the mechanisms of the above mentioned phenomena.

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